Salbutamol and exercise induced inspiratory flow limitation in asthmatic children

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Abstract

Objective: Exercise induced bronchoconstriction (EIB) is a frequent and specific symptom of childhood asthma featured by expiratory flow limitation. A recent study showed that exercise can also induce inspiratory flow limitation, independent of EIB. The aim of this study was to investigate whether salbutamol protects against exercise induced inspiratory flow limitation in asthmatic children.

Methods: The study had a prospective double-blind placebo-controlled randomized cross-over design with two exercise challenge tests preceded by the inhalation of 200µg salbutamol or placebo. Children 8-16 years old with documented exercise induced inspiratory flow limitation performed two exercise challenge tests (ECT's) to assess EIB. EIB was defined as a fall in forced expiratory volume in 1 second (FEV_1) ≥ 13% whereas inspiratory flow limitation was defined as a fall in mid inspiratory flow (MIF_{50}) ≥ 25%.

Results: 63% of the children (19/30) with exercise induced flow limitation showed an inspiratory flow limitation. Salbutamol significantly reduced the mean exercise induced fall in MIF_{50} in children with exercise induced inspiratory flow limitation compared to placebo (17.6% versus 24.9%, p=0.004).

Conclusions: There was a significant but inconsistent, individually variable protection of salbutamol against exercise induced inspiratory flow limitation observed in contrast to the consistent protective effect of salbutamol against EIB. A substantial number of the children with exercise induced flow limitation have an inspiratory flow limitation. Asthmatic children who experience persistent exercise induced asthmatic symptoms despite the use of (prophylactic) salbutamol, may suffer from an inspiratory flow limitation as a component of their asthma.

Key words:
exercise induced bronchoconstriction, pediatrics, bronchodilators, asthmatic children, salbutamol.

1. Introduction

Exercise induced bronchoconstriction (EIB) is a specific and common symptom of childhood asthma and of all asthma symptoms, considered to be the most detrimental on quality of life[1-3]. EIB is a sign of bronchial hyperresponsiveness (BHR) and featured by bronchial obstruction leading to expiratory flow limitation. Recent studies have shown that an exercise challenge test not only can induce EIB but also can induce inspiratory flow limitation[4-8]. Exercise induced inspiratory flow limitation is independent from EIB and also occurs after exercise. It is a different clinical entity than vocal cord dysfunction (VCD) which is accompanied by acute choking or an inspiratory stridor during exercise[4,5,7,8]. Inspiratory flow limitation is defined as a fall in mid inspiratory flow (MIF_{50}) of more than 25%[4,7,8]. Several studies have shown that inspiratory flow limitation can be induced by airway challenge tests other than exercise.

Exercise induces the release of mediators from inflammatory cells resident in the airway mucosa. These mediators are responsible for bronchial narrowing by activation of the inflammatory response in the asthmatic airway. Inhaled salbutamol stabilizes inflammatory cells and can therefore provide excellent protection[1,9]. The pathofysiology of exercise induced inspiratory flow limitation is unknown but inflammatory mediators released may be directly or indirectly involved. We hypothesize that salbutamol protects against inspiratory flow limitation implicating that inflammatory mediators are involved in the pathofysiology of inspiratory flow limitation.

The aim of this study was to investigate whether 200µg salbutamol protects against exercise induced inspiratory flow limitation in asthmatic children. The secondary aim was to investigate the relation between the protective effect of salbutamol against EIB and against exercise induced inspiratory flow limitation.

2. Materials & Methods

2.1 Patients

This study had a prospective double-blind placebo-controlled randomized cross-over design. Children 8 - 16 years with asthma, diagnosed by a pediatrician, were recruited from the outpatient clinic of the pediatric department of Medisch Spectrum Twente, Enschede (MST) from October 2013 to February 2014. Children were eligible if they demonstrated exercise induced inspiratory flow limitation with or without EIB during an exercise challenge test (ECT) within a period of two weeks prior to the study[10]. There were no restrictions to the use of medication, but children had to cease long acting bronchodilators or leukotriene antagonists 24 hours and short acting bronchodilators 8 hours before the ECT[5,11]. Children were excluded if they were admitted to the hospital or being prescribed systemic corticosteroids because of an exacerbation in the last eight weeks prior to the screening ECT.

2.2 Randomization and blinding

For randomization block sizes of 2 and 4 children were used. The randomization list was designed with the aid of a computerized randomization method (Windows version 6.0 randomization program "Rand.exe" by Steven Piantadosi) performed by an independent assistant. To ensure concealment of allocation, the randomization scheme was managed by an independent assistant (secretary of the pediatric department) and was not accessible to the researchers.

The administration of either salbutamol or a placebo prior to the exercise challenge test was inserted in a double-blind design and also the statistical analysis was performed blinded. TEVA pharmaceuticals provided the salbutamol and the placebo Autohalers®. Labeling to arrange the double-blinded design was performed under the conditions of good manufacturing practice by an external hospital. The inhalers were marked with codes which were kept in a sealed envelope by a secretary.

2.3 Exercise challenge test

To minimize anxiety which can lead to failed tests, the youngest children exercised on a jumping castle and the older children who were comfortable on a treadmill. For both exercise formats the same exercise challenge test guidelines were used[1,11].

During the four hours prior to the ECT's, children were not allowed to perform strenuous exercise.

After the screening ECT in which inspiratory flow limitation was assessed, the included children were randomized to perform two ECT's. The both ECTS's were preceded by the inhalation of 200µg salbutamol (Airomir® Autohaler) or placebo in a randomized order fifteen minutes prior to the ECT.

ECT's were planned with a minimum interval time of 2 days and a maximum of 14 days. ECT's and pulmonary function measurements were performed as previously described[5,11]. Children performed baseline spirometry measurements using a Microloop MK8 Spirometer (ML3535) according to the standard ERS protocol[12]. Koopman reference values were used to calculate the predicted value of FEV_1 [13]. After baseline spirometry children inhaled either 200µg salbutamol (Airomir® Autohaler) or placebo under supervision of the investigator to ensure correct technique. Fifteen minutes after inhaling children performed spirometry measurements again. Thereafter, children aged 8-10 years old jumped for a maximum of 6 minutes on a jumping castle in cold, dry air conditions (9.5-10 degrees Celsius and a relative humidity of 57-59%) in an indoor ice skating rink. Children aged 12-16 years old performed the ECT on a treadmill with a 10° slope (Trimline® 7150) under the same air conditions. Children aged 10-12 years old could choose between the two ECT formats. Heart rate was continuously monitored by a radiographic device (Garmin Forerunner 610) and the target was to achieve 80-90% of their maximum heart rate. An exercise induced fall in FEV_1 of ≥13% compared to baseline was considered as positive for EIB[10]. For a reliable measurement of the MIF_{50} the forced inspiratory vital capacity had to be within 7.5% of the forced expiratory vital capacity. A fall in MIF_{50} of ≥25% compared to baseline in more than one consecutive measurement was considered positive for an inspiratory flow limitation[5,7].

The degree of protection of salbutamol against exercise induced inspiratory flow limitation was assessed for each individual child based on the MIF_{50}. Children with a protection of fall in MIF_{50} of ≥50% were classified as responders to therapy i.e. if the MIF_{50} during the salbutamol ECT did not fall at least 50% compared to the placebo ECT, the child was considered as a responder. Children with a protection of fall in MIF_{50} of <50% were classified as non-responders to therapy.

2.4 Questionnaire

Children < 12 years old and their parents filled out the Childhood Asthma Control Test (C-ACT) to measure asthma control. Children ≥ 12 years old filled out the Asthma Control Test (ACT)[14,15].

2.5 Sample size calculation

A previous study investigating exercise induced inspiratory flow limitation in our clinic showed that 46% of asthmatic children (mean age 13.2 years old with a SD 2,2 years) had an exercised induced inspiratory flow limitation. The average fall in MIF_{50} was 25.8% (SD ±16.1%) after the exercise challenge test[5].

Our hypothesis was that inhalation of 200µg salbutamol prior to the ECT would offer a clinical relevant protection of 50% against inspiratory flow limitation. To document this significant difference in fall of MIF_{50} with a paired T-test a power calculation was performed.

Assuming an average fall in MIF_{50} of 25% (SD ±16%) in the placebo condition and an average fall in MIF_{50} of 12.5% (SD ±16%) when 200µg salbutamol is administered prior to the ECT, and assuming a significance level (alpha) of 0.05 and a power of 80%, 15 patients would be needed in a cross-over design.

2.6 Statistical analyses

Best values of spirometric measurements were used for statistical calculations. Results were expressed as mean values ± standard deviation (SD) for normally distributed data, as median (minimum; maximum) for not normally distributed data or as numbers with corresponding percentages if nominal or ordinal.

Within person changes in continuous variables (e.g. fall in FEV_1 or MIF_{50}) were analyzed with a paired T-test or a Wilcoxon signed rank, as appropriate. Between group differences in continuous variables were analyzed with an unpaired T-test (e.g. responders versus non-responders). Between-group comparisons of nominal or ordinal variables were performed by Chi-square tests (e.g. responders versus non-responders). To assess the correlation between two continuous variables (e.g. protection of salbutamol against EIB and inspiratory flow limitation) Spearman's rho was computed. A possible period or carry over effect was analyzed with the Hills and Armitage test. A 2 sided value of $P < 0.05$ was considered statistically significant. Data was analyzed with SPSS® for Windows® version 20 (IBM, Chicago, IL, USA) analytical software.

2.7 Ethical Considerations

This study was approved by the hospital ethics review board and the Central Committee on

Research Involving Human Subjects (CCMO) and registered in the Dutch Trial Register (http://www.trialregister.nl) number NTR4021. All children and parents/guardians received written patient information and provided written informed consent to participate in this study.

3. Results

Thirty children who showed exercise induced inspiratory flow limitation and/or EIB at the screening ECT within 2 weeks prior to the study were selected and screened. Eleven children (36.7%) with only EIB, but without exercise induced inspiratory flow limitation (fall in MIF_{50} of ≥25%) were excluded. Nineteen children (63.3%) with exercise induced inspiratory flow limitation with or without EIB were included. After inclusion 3 children were excluded from the study. One child was excluded because of unreliable lung function measurements, one due to an asthma exacerbation and one due to non-adherence with maintenance medication. Sixteen children composed the study group. Figure 1 shows the flow chart of inclusion.

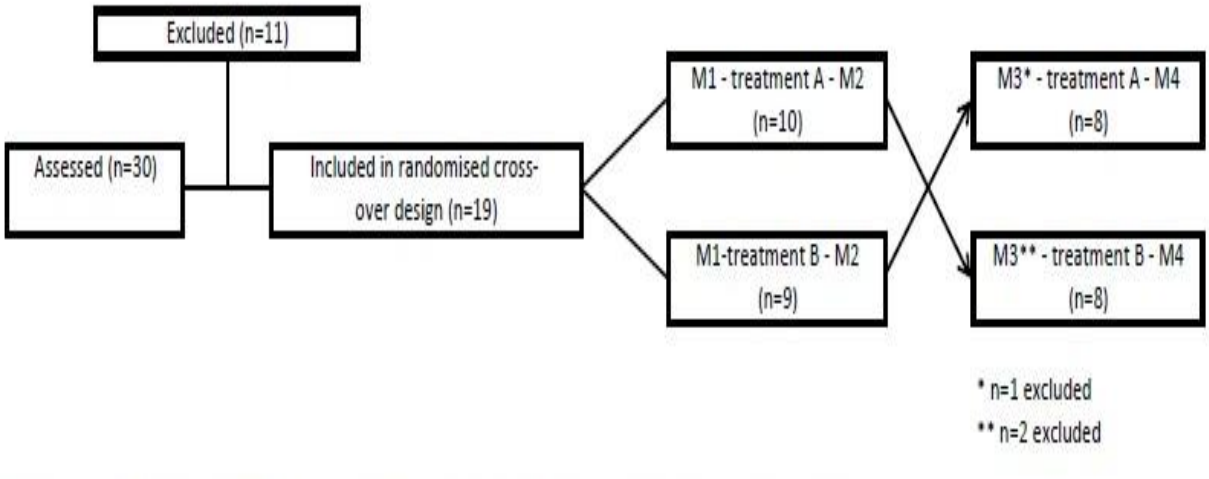


Figure 1: Flow chart of inclusion

Baseline characteristics of the 16 included children are shown in table 1. Hospitalization status indicates hospitalization due to an asthma exacerbation more than eight weeks before the start of the study.

Table 1: baseline characteristics

Number of children	16
Age in years (mean ± SD)	11.8 ± 2.2
Male gender (N, (%))	11 (68.8)
Hospitalisation before study (N, (%))	6 (37.5)
ECT format (N (%))	
Jumping castle	11 (68.8)
Treadmill	5 (31.2)
FEV_1 as % of predicted (mean ± SD)[a]	84.9 ± 9.8
Fall in FEV_1 in % (mean ± SD)	27.4 ± 17.1
Fall in MIF_{50} in % (mean ± SD)	39.1 ± 9.6
ICS (N, (%))	15 (93.8)
LTRAs (N, (%))	6 (37.5)
Allergy (N, (%))[b]	
Positive	9 (56.3)

Negative	4 (25)
Unknown	3 (18.8)
(C-)ACT ≤ 19 (N, (%)) [c]	8 (50)
Score (C)ACT (mean ± SD)	19.1 ± 4.9

SD: standard deviation; ECT: exercise challenge test; [a] FEV_1: forced expiratory volume in 1sec, percentage of predicted based on the reference values of Koopman et al[13]. MIF_{50}: maximal inspiratory flow at 50 percent of vital capacity. ICS: inhaled corticosteroids. LTRAs: leukotriene receptor antagonists. [b]Allergy: proven by blood test or skin prick test.
[c] (C-)ACT: (Childhood) Asthma Control Test: a score ≤19 points indicates uncontrolled asthma[14,15]

All 16 children performed two ECT's and achieved their target heart rate during their ECT's. Eleven children showed combined EIB with an inspiratory flow limitation, the other 5 children showed an isolated inspiratory flow limitation. The mean time to maximum fall in MIF_{50} was 4.5 min (± 3.9), while the mean time to maximum fall in FEV_1 was 3.6 min (± 2.7). There was a significant correlation between the fall in FEV_1 and the fall in MIF_{50} ($r=0.84$, $p < 0.001$).

No period effects or carry over effects were observed in this study (all p values > 0.43).

Salbutamol significantly reduced the mean exercise induced fall in MIF_{50} compared to placebo (17.6% versus 24.9%, p=0.004).

The FEV_1 value as percentage of predicted measured before administration of salbutamol did not significantly differ compared to placebo (4.1%; 95CI: 0.0%-8.4%; p = 0.06) or compared to the screening visit (2.9%; 95CI: -1.9%-7.7%; p = 0.22).
Exercise induced falls in MIF_{50} and FEV_1 at the screening ECT, after salbutamol and placebo, including their statistical differences are shown in table 2.

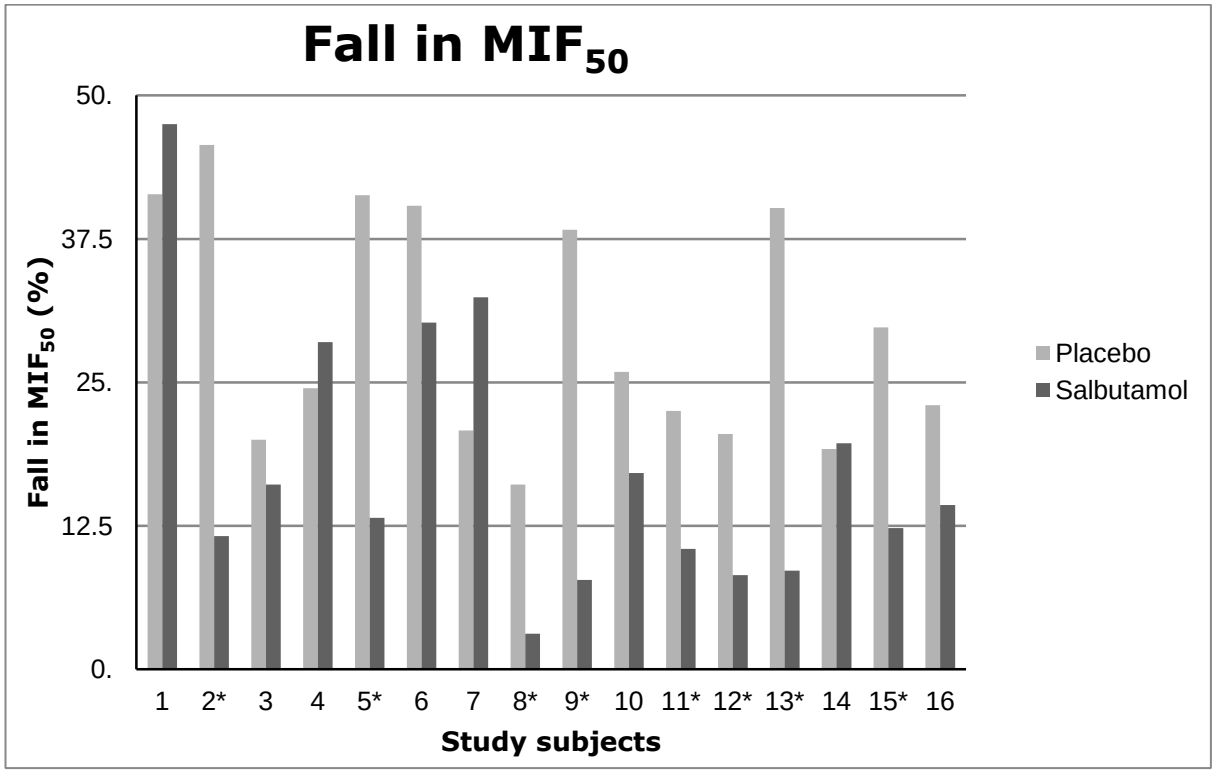
Table 2 Fall in MIF_{50} and FEV_1 at baseline, with placebo and with salbutamol

					Difference (95% CI); p-value		
		Baseline	Placebo	Salbutamol	Baseline-placebo	Baseline-salbutamol	Placebo- salbutamol
Total study group (n=16)	MIF_{50} (mean ± SD))	39.1 ± 9.6	29.4 ± 10.1	17.6 ± 11.6	9.7 (5.5-14.0) p <0.001	21.5 (15.7-27.3) p <0.001	11.8 (4.3-18.2) p=0.004
	FEV_1 (mean ± SD)	27.4 ± 17.1	22.5 ± 14.8	10.3 ± 7.9	4.9 (1.6-8.0) p=0.006	17.1 (8.1-25.9) p=0.001	12.2 (4.1-20.4) p=0.006
Isolated inspiratory flow limitation (n=5)	MIF_{50} (median (IQR))	32.5 (27.3-35.3)	22.5 (17.7-32.1)	10.5 (5.5-18.4)	10.0 p=0.080	22.0 p=0.043	12.0 p=0.080
Combined flow limitation (n=11)	MIF_{50} (median (IQR))	40.3 (32.4-49.8)	29.8 (20.8-41.3)	14.3 (11.6-30.2)	10.5 p=0.008	26.0 p=0.004	15.5 p=0.050
	FEV_1 (mean ± SD)	35.5 ± 14.4	29.5 ± 12.1	13.0 ± 7.9	6.0 (1.70-10.2) p=0.011	22.5 (10.7-34.2) p=0.002	16.5 (4.50-28.0) p=0.012

*Data expressed as mean values ± standard deviation, median (interquartile range (IQR)) or p value (95%CI). FEV_1: forced expiratory volume in 1 s. MIF_{50}: mid inspiratory flow at 50 percent of vital capacity. * In case of non normal data only difference with p-value is presented.*

The falls in MIF_{50} and FEV_1 separated by intervention per child are shown in figures 2 and 3.

Figure 2: Fall in MIF_{50} in percentage with placebo and with salbutamol for each individual child.



MIF_{50}: maximal inspiratory flow at 50 percent of vital capacity. * children with ≥50% protection on the MIF_{50} with salbutamol.

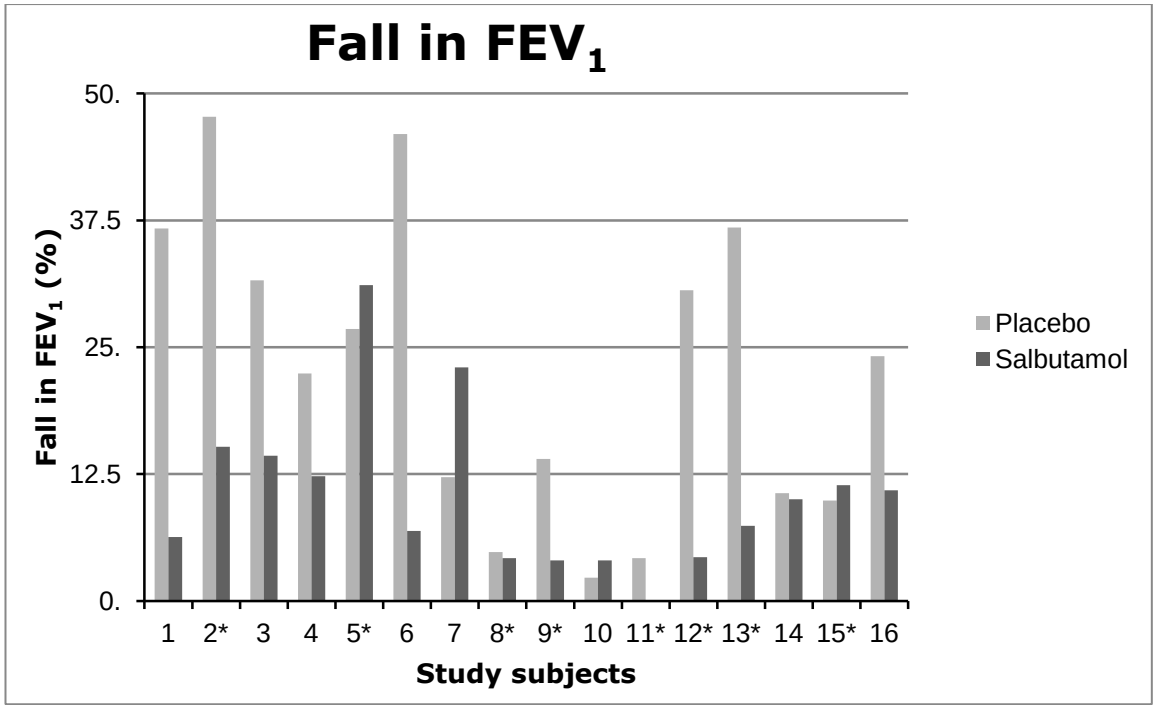


Figure 3: Fall in FEV_1 in percentage with placebo and with salbutamol for each individual child.

FEV_1: forced expiratory volume in 1 s. * children with ≥50% protection on the MIF_{50} with salbutamol.

Responders/ non-responders

As can be seen in figure 2, there were 8 responders to treatment with salbutamol against inspiratory flow limitation and 8 non-responders. The median percentage of protection ((% fall placebo - % fall salbutamol) / % fall placebo) of salbutamol against inspiratory flow limitation was 45.6% (IQR 2.9%-73.0%).

The characteristics of responders and non-responders against inspiratory flow limitation are shown in table 3. Characteristics were not significantly different between the responders and non-responders (all p values > 0.62), except for the higher use of leukotriene receptor antagonists in the group of non-responders (p=0.007).

Table 3: Characteristics of responders and non-responders

Patient characteristics	Responders	Non-responders
Number of children	8	8
Age, years (mean ± SD)	11.5 ± 2.0	12.0 ± 2.5
Boys (N, %)	6 (75)	5 (62.5)
FEV_1 as % of predicted [a] (mean ± SD)	86.5 ± 12.5	83.3 ± 6.5
Fall in FEV_1 in % at baseline (mean ± SD)	25.9 ± 16.6	28.9 ± 18.6
Fall in MIF_{50} in % at baseline (mean ± SD)	37.0 ± 9.9	41.1 ± 9.3
Hospitalisation before study (N, %)	3 (37.5)	3 (37.5)

ICS (N, %)	7 (87.5)	8 (100)
LTRAs (N, %)	0	6 (75)
Allergy (N, %) [b]		
proven	5 (62.5)	4 (50.0)
unknown	1 (12.5)	2 (25.0)
(C)ACT ≤ 19 (N, %) [c]	3 (37.5)	5 (62,5)
Score (C)ACT (mean ± SD)	19.6 ± 5.1	18.6 ± 5.0

Data expressed as mean ± SD, median (IQR) or numbers (percentage). [a]FEV_1: forced expiratory volume in 1sec, percentage of predicted based on the reference values of Koopman et al[13]. $MIF_{50:}$ maximal inspiratory flow at 50 percent of vital capacity. ICS: inhaled corticosteroids. NCS: nasal corticosteroid. LTRAs: leukotriene receptor antagonists. [b] Allergy: proven by radioallergosorbent test or blood test. [c] (C)ACT: (Childhood) Asthma Control Test: a score ≤19 points indicates uncontrolled asthma[14,15].

The screening ECT showed 11 children with a combined inspiratory and expiratory flow limitation. These children were analyzed for the relation between the protective effect of salbutamol against an inspiratory flow limitation and EIB.

No correlation was found between the protection of salbutamol against fall in FEV_1 and against fall in MIF_{50} in comparison to placebo (r= 0.21; p = 0.43).

5. Discussion

An inconsistent, individually variable protection of salbutamol against exercise induced inspiratory flow limitation was observed, in contrast to the consistent protective effect of salbutamol against EIB. This study confirmed that a substantial number of asthmatic children with exercise induced flow limitation have an inspiratory flow limitation which is independent from EIB.

This study showed the same prevalence of exercise induced inspiratory, expiratory and combined flow limitation as other studies investigating flow limitation after airway challenge in asthmatic children and adults.
To our knowledge, this is the first study to analyze the protection of salbutamol against inspiratory flow limitation in asthmatic patients. One study found a significant reduction of metacholine induced inspiratory flow limitation with a combined treatment of nasal corticosteroids, pseudoephedrine and antibiotics in children[8].

Exercise induced hyperventilation dries the airway epithelium and leads to hyperosmolarity of the airway surface fluid, triggering residential mucosal mast cells to release inflammatory mediators such as histamine[1,16]. It is assumed that the bronchoprotective effect of salbutamol in EIB is largely attained by its stabilizing effect on beta 2 receptors on mast cells[1,9]. Exercise also cools the airways, that rapidly rewarm and congest when exercise induced hyperventilation ceases. Both cooling and drying mainly occur in the larger airways. As only a mild protective effect of salbutamol against exercise induced inspiratory flow limitation was found, in contrast to the consistent effect on EIB, we speculate that the role of inflammatory mediators is not as important in the pathofysiology of exercise induced inspiratory flow limitation as in EIB. Perhaps rebound rewarming after exercise of the hyperplastic vascular bed present in asthmatic airways can lead to congestion and obstruction of the larger airways leading to an inspiratory flow limitation. Asthma is not in all patients confined to conductive and small airways and possibly the inspiratory flow limitation reflects the presence of airway inflammation in the larger airways.
Asthmatic children who experience persistent exercise induced asthmatic symptoms despite the use of (prophylactic) salbutamol, may suffer from an inspiratory flow limitation as a component of their asthma.

Exercise induced inspiratory flow limitation can be induced by vocal cord dysfunction (VCD). However, the inspiratory flow limitation we observed progressed after ceasing exercise and was not accompanied with acute choking or an inspiratory stridor, which strongly suggests another cause than VCD[17-20]. Moreover VCD is relatively rare in this young age group whilst an inspiratory flow limitation was observed in the majority of children.

In our population 19.4% of the children were not able to perform reliable and duplicated inspiratory flow-volume loops. This is similar to Tomalek et al. et al. who showed that 23% of healthy children in a similar age group could not perform acceptable inspiratory flow-volume loops[21]. According to ERS criteria volume loops need to be repeated to obtain a reliable value.

The main strength of this study is the prospective double-blind placebo-controlled randomized cross-over design. Also, a short time period between the two interventions was pursued (<1 week) and all tests were carried out by the same investigator in standardized air conditions. None of the children quitted the ECT's prematurely.
A limitation of our study is that due to the tight time schedule of obtaining flow volume loops after exercise, not all children were able to perform comparable duplicated inspiratory volume loops.
Another limitation is the administration of 200µg salbutamol which could have been a too low dose to result in a clinical effect in all children with exercise induced inspiratory flow limitation.

More research is necessary to analyze the pathophysiological basis of exercise induced inspiratory flow limitation. A study investigating the protection of inhaled vasoconstrictive agents, such as alpha agonists, against exercise induced flow limitation to evaluate the contribution of vascular phenomena to an exercised induced inspiratory flow limitation and EIB could be of additional value.

6. Conclusions

This study showed an inconsistent, individually variable protection of salbutamol against exercise induced inspiratory flow limitation in contrast to the consistent protective effect of salbutamol against EIB. A substantial number of asthmatic children with exercise induced flow limitation have an inspiratory flow limitation which is independent from EIB was found.

Asthmatic children who experience salbutamol resistant exercise induced symptoms may suffer from an inspiratory flow limitation, which can be identified in an ECT with measurement of both in and expiratory flow volume loops.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Reference List

1. Randolph C. Pediatric exercise-induced bronchoconstriction: contemporary developments in epidemiology, pathogenesis, presentation, diagnosis, and therapy. *Curr Allergy Asthma Rep.* 2013;13:662-671.

2. Croft D, Lloyd B. Asthma spoils sport for too many children. *Practitioner.* 1989;233:969, 971.

3. Merikallio VJ, Mustalahti K, Remes ST, Valovirta EJ, Kaila M. Comparison of quality of life between asthmatic and healthy school children. *Pediatr Allergy Immunol.* 2005;16:332-340.

4. Bucca C, Rolla G, Brussino L, De R, V, Bugiani M. Are asthma-like symptoms due to bronchial or extrathoracic airway dysfunction? *Lancet.* 1995;346:791-795.

5. Driessen JM, van der Palen J, van Aalderen WM, de Jongh FH, Thio BJ. Inspiratory airflow limitation after exercise challenge in cold air in asthmatic children. *Respir Med.* 2012;106:1362-1368.

6. Joos GF, O'Connor B, Anderson SD, Chung F, Cockcroft DW, Dahlen B, et al. Indirect airway challenges. *Eur Respir J.* 2003;21:1050-1068.

7. Kelso JM, Enright PL, Scanlon PD, O'Connell EJ, Sachs MI. Effect of inhaled methacholine on inspiratory flow. *Chest.* 1990;98:1426-1429.

8. Turktas I, Dalgic N, Bostanci I, Cengizlier R. Extrathoracic airway responsiveness in children with asthma-like symptoms, including chronic persistent cough. *Pediatr Pulmonol.* 2002;34:172-180.

9. Russo C, Zeng D, Prosperini G, Spicuzza L, Guarino F, Polosa R. Effect of salbutamol on nasal symptoms and mast cell degranulation induced by adenosine 5' monophosphate nasal challenge. *Clin Exp Allergy.* 2005;35:1192-1196.

10. Vilozni D, Bentur L, Efrati O, Barak A, Szeinberg A, Shoseyov D, et al. Exercise challenge test in 3- to 6-year-old asthmatic children. *Chest.* 2007;132:497-503.

11. van Leeuwen JC, Driessen JM, de Jongh FH, Anderson SD, Thio BJ. Measuring breakthrough exercise-induced bronchoconstriction in young asthmatic children using a jumping castle. *J Allergy Clin Immunol.* 2013;131:1427-1429.

12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J.* 2005;26:319-338.

13. Koopman M, Zanen P, Kruitwagen CL, van der Ent CK, Arets HG. Reference values for paediatric pulmonary function testing: The Utrecht dataset. *Respir Med.* 2011;105:15-23.

14. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the Childhood Asthma Control Test. *J Allergy Clin Immunol.* 2007;119:817-825.

15. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol.* 2004;113:59-65.

16. Anderson SD. Exercise-induced bronchoconstriction in the 21st century. *J Am Osteopath Assoc.* 2011;111:S3-10.

17. Kenn K, Balkissoon R. Vocal cord dysfunction: what do we know? *Eur Respir J.* 2011;37:194-200.

18. McFadden ER, Jr., Zawadski DK. Vocal cord dysfunction masquerading as exercise-induced asthma. a physiologic cause for "choking" during athletic activities. *Am J Respir Crit Care Med.* 1996;153:942-947.

19. Rundell KW, Spiering BA. Inspiratory stridor in elite athletes. *Chest.* 2003;123:468-474.

20. Watson MA, King CS, Holley AB, Greenburg DL, Mikita JA. Clinical and lung-function variables associated with vocal cord dysfunction. *Respir Care*. 2009;54:467-473.

21. Tomalak W, Radlinski J, Pogorzelski A, Doniec Z. Reference values for forced inspiratory flows in children aged 7-15 years. *Pediatr Pulmonol.* 2004;38:246-249.

